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March 2, 1995

Document Processing Center (7407)
(Attn.: Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Contains No CBI

RE.: **TSCA Section 8(e) Notice; Mixture of CAS Nos. 71839-88-8 and 85203-44-7**

Dear Section 8(e) Coordinator:

This letter and the enclosed study contain no Confidential Business Information.

In accordance with EPA's March 16, 1978, policy statement on Section 8(e) reporting under the Toxic Substances Control Act and EPA's June 1991 TSCA Section 8(e) Reporting Guide, Ciba wishes to bring to your attention acute toxicity observed in carp with an approximately 50/50 mixture of CAS Nos. 71839-88-8 and 85203-44-7. Chemically, CAS No. 71839-88-8 is Cobaltate(1-), bis(2,4-dihydro-4-[(2-hydroxy-5-nitrophenyl)azo]-5-methyl-1-phenyl-3H-pyrazol-3-onato(2-)]-, sodium. CAS No. 85203-44-7 is Amines, C_{12-18}-alkyl, bis[2,4-dihydro-4-[(2-hydroxy-5-nitrophenyl)azo]-5-methyl-2-phenyl-3H-pyrazol-3-onato (2-)]cobaltate(1-). The subject chemical mixture is an imported pigment that is sold commercially in the United States. It is used for organic solvent based ink products.

Acute toxicity was determined in carp in accordance with OECD Guideline 203 under static conditions. The test material, dissolved in ethanol, was added to the aquaria at various aqueous concentrations and was considered less stable as concentrations decreased. The nominal 96-hour LD_{50} was estimated to be about 0.1 mg/l, under these artificial conditions. A copy of the final report, entitled "96-Hour Acute Toxicity Study (LC_{50}) in the Carp (Static)," is enclosed.

In a separate modified Sturm test, the test material does not appear to be readily biodegradable. Although we do not have an experimentally determined value for the octanol/water partition coefficient for the mixture in question, a recently obtained value, **calculated** by our parent company in Basel, Switzerland, showed the Log P to be 8.66 (Fragment method, C. Hansch and A. Leo, Computer program CLOP 3.4). In spite of this chemical mixture's potential to bioaccumulate, as evidenced by the modified Sturm test result and the high calculated Log P, Ciba is not aware of any significant potential for widespread exposure. Ink makers routinely handle wastes of the subject mixture as hazardous waste, which is subsequently incinerated. (Cobalt compounds are regulated as hazardous wastes

3/15/95

under Section 313 of SARA Title III.) It is highly unlikely that even small quantities of this product mixture would find its way into aqueous environments, since the mixture would color the water intensely, and would alarm any POTW, or other waste stream operator. Additionally, the mixture is not water soluble.

Ciba is submitting this environmental effects information under TSCA Section 8(e) out of an excess of caution. We believe it may not be subject to 8(e) reporting, particularly if Part V(b)(2) and (3) of EPA's 1978 8(e) guidance are applied strictly. Under Part V(b)(2) of the guidance, reporting would be required if the following criteria are met: a) pronounced bioaccumulation as evidenced by an n-octanol water partition coefficient greater than 25,000, b) potential for **widespread** exposure, and c) any non-trivial adverse effect. Because "widespread" has not yet been defined by EPA, we do not know whether there is a potential for widespread exposure. Under Part V(b)(3), reporting would be required if the following criterion is met: any non-trivial adverse effect associated with a chemical **known** either a) to have bioaccumulated **or** b)to be **widespread** in environmental media. The subject chemical mixture is not known to have bioaccumulated and, as discussed above, the term "widespread" has not been defined by EPA.

As you know, EPA has suspended Part V(b)(1) of its 1978 guidance, which deals with **widespread** and previously unsuspected distribution in environmental media. Since the term, "widespread," is not yet defined by EPA, we are concerned that it may be subsequently defined in Part V(b) in such a manner as to require reporting of the information in this submission. We are therefore submitting the information now, and request EPA to inform us whether the information is, indeed, subject to immediate reporting under TSCA Section 8(e).

Ciba will include the acute carp toxicity finding on the Material Safety Data Sheet.

Please contact the undersigned if you need any additional information.

Very truly yours,

A. Di Battista

Anthony Di Battista

AD02275b.WP/dch
Attachment

RCC NOTOX
Research and Consulting Company b.v.

R C C N O T O X B.V.

RCC NOTOX PROJECT 007919

ORASOL ORANGE G

(RCC NOTOX SUBSTANCE 2195)

96-HOUR ACUTE TOXICITY STUDY (LC50)

IN THE CARP

(STATIC)

REPORT

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Telex 50730 CTEAMNL

REPORT APPROVAL

Study Director: Drs. M. Bogers

Date: 31/7/1990

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SUMMARY

ORASOL ORANGE G 96-hour Acute Toxicity Study (LC50) in the Carp.

Determination of stability in a pretest revealed that at a nominal concentration of 10 mg/l, ORASOL ORANGE G was stable under test conditions for at least 96 hours.

Ten fish per concentration, mean length: 2.1 cm, were exposed for 96 hours in a static system to a concentration range of nominally 0.010 to 0.32 mg/l forming a geometric progression with factor 1.8. Because of the limited solubility of ORASOL ORANGE G in water, stock solutions were made up in ethanol and an extra group of fish was exposed to an ethanol-control. Actual concentrations were based on the results of analysis of the samples taken during the main study.

After 48 hours of exposure all fish had died at 0.32 mg/l and 7 out of 10 fish at 0.18 mg/l. At the end of the exposure period 100% mortality was recorded at 0.18 mg/l and 20% at 0.10 mg/l, whereas no mortality was observed at the other concentrations

Analysis of the samples taken during the main study revealed that no accurate results could be obtained due to the observed variability in the analytical method. During the exposure period the actual concentration analyzed decreased significantly.

During exposure the LC50 decreased with time from nominally 0.27 mg/l at 24 hours to 0.12 mg/l at 72 hours. At 96 hours no exact LC50 value could be calculated but based on the other data the 96h-LC50 was estimated to be between nominally 0.10 and 0.12 mg/l with 0% mortality at 0.056 mg/l and 100% mortality at 0.18 mg/l.

PREFACE

GENERAL

Title	ORASOL ORANGE G, 96 hour Acute Toxicity Study (LC50) in the Carp
Sponsor	CIBA GEIGY CH-4002 BASEL SWITZERLAND
Monitoring Scientist	Dr. A. von Schulthess
Testing Facility	RCC NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch, The Netherlands
RCC NOTOX Project	007919
RCC Project	228543
Test substance	ORASOL ORANGE G
Test system	Carp (Cyprinus carpio)

PROJECT

Aquatic toxicology:	
Study Director	Drs. M. Bogers
Technical Head	G.J.Z. Gols
Analytical chemistry:	
Principal Scientist	Ir. J.M. Cardinaals

SCHEDULE

Aquatic toxicology:	
Start of the range finding	October 2, 1989
Completion of the main study	October 20, 1989
Analytical chemistry:	
Start of analysis	September 5, 1989
Completion of analysis	October 20, 1989

QUALITY ASSURANCE STATEMENT

RCC NOTOX Project Number	007919
Test substance	ORASOL ORANGE G
Study Director	Drs. M. Bogers
Title	ORASOL ORANGE G, 96 hour Acute Toxicity Study (LC50) in the Carp.

Study procedures were periodically inspected and this report was audited by the Quality Assurance. The dates are given below.

Dates of QAU Inspections / Audits	Dates of reports to the Study Director and Management
23-05-1989	23-05-1989
03-10-1989	03-10-1989
31-01-1990	31-01-1990

Manager, Quality Assurance Unit C.J. Mitchell B. Sc.

Date: 05-02-90

STATEMENT OF GLP COMPLIANCE

RCC NOTOX Project Number	007919
Test substance	ORASOL ORANGE G
Study Director	Drs. M. Bogers
Title	ORASOL ORANGE G, 96 hour Acute Toxicity Study (LC50) in the Carp.

To the best of my knowledge and belief the main study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

OECD Principles of Good Laboratory Practice, Paris France, adopted May 12, 1981.

Food and Drug Administration - Non-clinical laboratory studies: Good Laboratory Practice Regulations, U.S.A. Federal Register, Vol. 43, no. 247, December 22, 1978 and subsequent amendments.

Environmental Protection Agency - Pesticide Programs and Toxic Substances Control; Good Laboratory Practice standards; U.S.A., Federal Register, Vol. 54 No. 158, August 17, 1989.

Study Director Drs. M. Bogers

Date: 31/01/1990

GUIDELINES

The study procedure described in this report is based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD guidelines for the testing of Chemicals, guideline No. 203: "Fish Acute toxicity Test", Adopted April 4, 1984

European Economic Community (EEC),EEC directive 84/449, Methods for the determination of Ecotoxicity, Publication No. L251, C-1:"Acute Toxicity for Fish", adopted September, 1984.

Environmental Protection Agency (EPA), Pesticide Assessment Guidelines, Subvision E, Hazard Evaluation: Wildlife and Aquatic Organisms, No. 72-1, Acute Toxicity for Freshwater Fish, Office of Pesticide and Toxic Substances, EPA 540/9-82-024, Washington, USA, October 1982.

Biologische Bundesanstalt für Land- und Forstwirtschaft, BBA Merkblatt Nr 33, September 1979 (Federal Republic of Germany).

SUMMARY OF PROTOCOL AMENDMENTS

1. Due to aeration during the test, the pH level recorded during the study ranged from 7.8 to 8.9 and thus exceeded the optimal range of 6.0 to 8.5.
2. The final 96-hour acute toxicity study was a LC50 study instead of a limit study, because in the pilot the test substance appeared to induce mortality in carps below 1000 mg/l.
3. The analytical chemistry was performed by RCC NOTOX with Ir. J.M. Cardinaals as the principal scientist.
4. Dissolved oxygen content, temperature and pH were measured in all vessels prior to addition of the fish and daily during the exposure period.
5. For practical reasons the first recording of mortality and other effects was performed after 5 hours of exposure.
6. Instead of weighing all fish of each tank, a representative number of fish (10) belonging to the batch, from which fish were used for the present study, were weighed and measured prior to the start of the test.

ARCHIVING

RCC NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.

PURPOSE

The purpose of the study is to evaluate the test substance for its ability to generate acute toxic effects in Cyprinus carpio during a test period of 96 hours and, if possible, to determine the 96h-LC50.

MATERIALS AND METHODS

TEST SYSTEM

Species	Carp (Cyprinus carpio), Teleostei, Cyprinidae) (Linnaeus, 1758)
Source	Zodiac, proefacc, "De Haar Vissen", L.U. Wageningen, the Netherlands.
Characteristics	Pathogenic-free F1 from a single parent-pair.
Reason for selection	This system has been selected as an internationally accepted species and is recommended by the guidelines referred to.
Acclimation period	After delivery, the fish were held in 100-liter tanks for at least 14 days.
Medium	Filtered and aerated tap water is supplied continuously. A certificate of contaminant analysis is attached to the report.
Temperature	23 ± 2°C
Feed	Artemia or Trouvit OO.
Frequency	Once a day.
Adaption	Prior to the testing, the fish were adapted to the test medium without test substance for at least seven days following a 48 hour settling in period.
Validity of batch	In the batch of which fish were used for the test, mortality during seven days prior to the start of the test was less than 5%.

TEST SUBSTANCE

Identification	: ORASOL ORANGE G
Description	: Orange solid
Batch Number	: 216096.89
Purity/composition	: 45-55% ORASOL Orange G new type Comp. I 55-45% ORASOL Orange G new type Comp. II
Instructions for test article storage	: At 20°C in the dark
Stability of test article	: Stable, > 5 years at storage conditions
Expiry date	: 31-12-1993
Stability in vehicle	: Stable for at least 2 hours in water
Safety precautions	: Gloves, goggles and face mask will be sufficient to ensure personnel health and safety

TEST PROCEDURE

Identification	The vessels were individually identified by means of adhesive labels as described in detail in RCC NOTOX's Standard operating Procedures.
Test type	LC-50, Static
Test duration	96 hours
Test vessels	all-glass
Test medium	Tap-water, continuously aerated.
Fish length	2.1 cm; s.d. = 0.20 (n=10)
Mean fish weight	0.20 g; s.d. = 0.023 (n=10)
Number of fish	10 fish per concentration
Loading	1 g of fish per litre of test medium, i.e. 10 fish per 2 litres of test medium.
Light	16 hours photoperiod daily
Room temperature	22°C - 24°C
Feeding	No feeding from 24 hours prior to the test and during the total test period

TEST CONCENTRATIONS

Concentration range	Based on the results of the pretest: i.e. nominally: 0.010, 0.018, 0.032, 0.056, 0.10 0.18 and 0.32 mg/l.
- Nominal concentration	Expressed as weight of test substance per volume of water based on amount of test substance added to the test media.
- Actual concentration	Based on the results obtained from the chemical analysis performed during the main study.
Controls	Blank: test medium without test substance or other additives. Ethanol control: test medium with 0.032 ml ethanol/l tapwater.

PREPARATION OF TEST MEDIA

Treatment stock solutions:
A stock solution of 10 g/l in ethanol was prepared by addition of 0.993 g of the test substance to 100 ml ethanol. From this solution 1 ml was diluted in 1000 ml of tap water (10 mg/l). This second solution was used to prepare the different test concentrations.

Introduction of fish	Within approximately 10 minutes after preparation of the test media.

RECORDINGS AND MEASUREMENTS

Mortality and other effects	5, 24, 48, 72 and 96 hours after start of exposure.
Fish size and weight	10 fish of the batch, from which fish were used for the present study, were weighed and measured prior to the start of the test.
Dissolved oxygen content and pH	In all vessels prior to addition of the fish and daily thereafter.
Water temperature	In the blank control vessels every day of the test using a thermometer.

ANALYSIS OF TEST CONCENTRATIONS

Stability of the test substance under test conditions was examined by analysis of samples taken during a range finding test. Duplicate 25 ml samples were taken from a vessel treated with 10 mg/l without the presence of fish. Furthermore, duplicate 25 ml samples were also taken from the vessels treated with 1 and 0.1 mg/l with fish present in the test media. These samples were taken at t= 0, 24 and 96 hours.
Further analysis during the definite study was performed in duplicate, using samples from three different concentrations: 0.010, 0.056 and 0.32 mg/l. In addition samples were taken from the blank.

Sampling:	Frequency	In duplicate from the approximate centre of the vessels at t= 0, 2, 24, 48 and 96 hours.
	Volume	5 ml (an extra 100 ml sample was taken for additional analysis if necessary)
	Storage	All samples were stored at -20 °C until analysis and the additional 100 ml samples are stored for three months after delivery of the report, pending on the decision of the sponsor for additional analyses.

DATA HANDLING

Definitions:

- Mortality	Fish were considered to be dead when no reaction was observed after touching the caudal peduncle, combined with the absence of visible breathing movements.
- LC50	The LC50 is the concentration killing 50% of the fish after a certain period of exposure.

The LC50 was determined using:
The maximum likelihood estimation method with the probits of the percentages of dead fish as function of the logarithms of the corresponding concentrations (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition)

RESULTS

RANGE-FINDING TEST

In the range finding test 100% mortality was recorded at nominally 1 mg/l and 10 mg/l after 6 hours of exposure. At 0.1 mg/l (nominal) 2 out of 5 fish had died after 96 hours of exposure.

STABILITY OF TEST SUBSTANCE UNDER TEST CONDITIONS

The results of determination of stability are described in the appended Analytical Report.

Analysis of the samples taken during the range finding revealed that at a concentration of 10 mg/l, ORASOL ORANGE G was stable under test conditions for at least 96 hours and at this level the concentration analyzed was in agreement with the nominal concentration. At t=0 the actual concentrations at 1 mg/l and 0.1 mg/l were ± 10-30% and 70% lower than the nominal concentrations, respectively. Furthermore the concentrations analyzed showed a tendency to decline in time.

MAIN STUDY: MORTALITY AND OTHER EFFECTS

The mortality data of the main study are presented in Tables 1 and 2.

After 48 hours of exposure all fish had died at 0.32 mg/l and 7 out of 10 fish at 0.18 mg/l. At the end of the exposure period 100% mortality was recorded at 0.18 mg/l and 20% at 0.10 mg/l, whereas no mortality was observed at the other concentrations or the controls.
No other effects than mortality were recorded.

MAIN STUDY: EXPERIMENTAL CONDITIONS

The results of pH and oxygen measurements are presented in Tables 4 and 5.

The pH ranged from 7.8 to 8.8.

Oxygen concentration in the test media was found to be > 5 mg/l for all measurements performed during the main study.

The temperature of the test medium measured in the blank control varied from 21.5 to 22.5°C.

MAIN STUDY: ACTUAL VERSUS NOMINAL CONCENTRATIONS

The results of analysis of the samples taken during the main study are described in the appended Analytical Report.

Analysis of the samples revealed that no accurate results could be obtained due to the observed variability in the analytical method. The concentration of ORASOL ORANGE G analyzed in most samples was significantly lower than the nominal concentration, with no detectable levels of ORASOL ORANGE B at 0.010 mg/l (<0.005).
During the exposure period the actual concentration decreased significantly resulting in 30-60% of the nominal concentration left in the samples taken from nominally 0.32 mg/l and no detectable levels of ORASOL ORANGE B in the samples taken from 0.056 mg/l after 96 hours.

CALCULATION OF LC50

During exposure the LC50 decreased with time from nominally 0.27 mg/l at 24 hours to 0.12 mg/l at 72 hours (see Table 3). At 96 hours no exact LC50 value could be calculated but based on the other data the 96h-LC50 was estimated to be between nominally 0.10 and 0.12 mg/l with 0% mortality at 0.056 mg/l and 100% mortality at 0.18 mg/l.

VALIDITY

Since no mortality or other effects were observed in the blank and the ethanol-control the results recorded in the main study are considered valid.

CONCLUSION

Under the conditions of the present test ORASOL ORANGE G appeares to induce mortality at nominally 0.10 mg/l and higher. The nominal 96h-LC50 for fish exposed to ORASOL ORANGE G is estimated to be between 0.10 and 0.12 mg/l with 0% mortality at 0.056 mg/l and 100% mortality at 0.18 mg/l.

TABLE 1: Incidence of mortality observed in the main study

Nominal concentration (mg/l)	Number of fish exposed	Number of dead fish recorded at different points in time after start of exposure 5h	24h	48h	72h	96h
blank	10	0	0	0	0	0
ethanol-control	10	0	0	0	0	0
0.010	10	0	0	0	0	0
0.018	10	0	0	0	0	0
0.032	10	0	0	0	0	0
0.10	10	0	0	1	1[a]	0[a]
0.18	10	0	1	6	2[a]	1[a]
0.32	10	0	7	3	-	-

[a] Surviving fish were discoloured orange due to the presence of the test substance..

TABLE 2: Total rate of mortality recorded at the end of the main study.

Nominal concentration (mg/l)	total number of fish	number of dead fish	total rate of mortality
blank	10	0	0%
ethanol-control	10	0	0%
0.010	10	0	0%
0.018	10	0	0%
0.032	10	0	0%
0.056	10	0	0%
0.10	10	2	20%
0.18	10	10	100%
0.32	10	10	100%

TABLE 3: LC50 values and related parameters calculated from the results of the main study.

Nominal conc.(mg/l)	Cumulative mortality (%) 24h	48h	72h	96h
0.056		0	0	0
0.10	0	10	20	20
0.18	10	70	90	100
0.32	70	100	100	
LC50 (mg/l)	0.27	0.15	0.12	0.10-0.12
95%-confidence interval	0.22-0.35	0.12-0.18	0.10-0.15	[a]

[a] The LC-50 could not be calculated according to Finney.

TABLE 4: pH levels after various time intervals

Nominal conc. (mg/l)	pH-values 0h	24h	48h	72h	96h
blank	7.8	8.8	8.6	8.6	8.7
ethanol-contr.	7.9	8.8	8.6	8.8	8.8
0.010	8.0	8.8	8.6	8.7	8.7
0.018	8.0	8.8	8.8	8.7	8.8
0.032	8.0	8.4	8.8	8.7	8.7
0.056	8.0	8.8	8.7	8.7	8.8
0.10	8.0	8.8	8.7	8.8	8.8
0.18	8.0	8.8	8.7	8.7	8.8
0.32	8.0	8.8	-a	-	-

a The pH measured after all fish had been found dead was 8.4.

TABLE 5: Oxygen concentrations after various time intervals

Nominal conc. (mg/l)	Actual conc. (mg/l)	Oxygen concentration (mg/l) 0h	24h	48h	72h	96h
blank		9.3	8.5	8.5	8.2	8.0
ethanol-contr.		9.3	8.2	8.0	8.2	8.1
0.010		9.4	8.4	7.9	8.3	8.1
0.018		9.4	8.4	8.6	8.3	8.2
0.032		9.4	6.6	8.6	8.3	8.3
0.056		9.5	8.2	8.4	7.5	8.1
0.10		9.3	8.6	8.3	7.5	8.3
0.18		9.3	8.5	8.6	8.1	8.3
0.32		9.3	8.3	-a	-	-

The oxygen concentration level measured after all fish had been found dead was 8.8.

ANALYSIS OF TAP WATER

Date of sampling : August 8 and 15, 1989
Sample numbers : 051439 and 106531
Principal scientist: Ir. P.E.M. Pieters, B.C.O. B.V., Breda, The Netherlands.

COMPONENT	ANALYSIS
Metals:	
Copper[a]	15 µg/l
Arsenic	< 2 µg/l
Cadmium	< 0.1 µg/l
Calcium	81000 µg/l
Chromium	< 1 µg/l
Iron	< 30 µg/l
Lead	< 15 µg/l
Magnesium	8300 µg/l
Mangaan	< 3 µg/l
Mercury	< 0.02 µg/l
Selenium	< 2 µg/l
Zinc	11 µg/l
EOX	< 0.10 µg/l
Polycyclic Aromatic Hydrocarbons:	
Naphthalene	< 0.2 µg/l
Phenanthrane	< 0.01 µg/l
Anthracene	< 0.01 µg/l
Pyrene	< 0.01 µg/l
Fluoranthrene	< 0.01 µg/l
Benzo(a)pyrene	< 0.005µg/l
Acenaphthylene	< 0.05 µg/l
Acenaphthene	< 0.05 µg/l
Fluorene	< 0.01 µg/l
Benzo(a)antracene	< 0.01 µg/l
Chrysene	< 0.01 µg/l
Benzo(b)fluor.	< 0.005µg/l
Benzo(k)fluor.	< 0.005µg/l
Dibenzo(ah)antracene	< 0.01 µg/l
Benzo(ghi) per.	< 0.01 µg/l
Indenol23cdPyrene	< 0.01 µg/l
Clarity	0.55 FTE
Colour intensity	21 Pt/Co-scale mg/l Pt
Totale aerobic germ-count (37°C)	<1 x 10E0 cfu/ml
Coliforms	<1 x 10E0 cfu/250 ml
Enterobacteria	<1 x 10E0 cfu/250 ml
Others: Hardness[a]:	2.2 mmol/l
Nitrate:	4400 µg/l
Nitrite:	<100 µg/l

Since no chlorine was present, declorination of tap water was not necessary.

[a] Tap water was sampled at the same point at which tap water was supplied for the present toxicity study. Copper content and hardness were measured in these samples.
[b] The other components were analysed in samples taken from the water supply of the Animal House.

R C C N O T O X B. V.

RCC NOTOX PROJECT 007919

ORASOL ORANGE G

(RCC NOTOX substance 2195)

96-HOUR ACUTE TOXICITY STUDY (LC50) IN THE CARP

DETERMINATION OF TEST CONCENTRATIONS

ANALYTICAL REPORT

Hambakenwetering 7
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Phone (0)73 - 41 95 75
Telefax (0)73 - 41 85 43
Telex 50730 CTEAMNL

REPORT APPROVAL

I, the undersigned declare that the study reported here has been carried out according to the agreed protocol and this report contains an accurate description of the results.

PRINCIPAL SCIENTIST: Ir. J.M. Cardinaals

.....

date: February 01, 1990

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SUMMARY

96-hour acute toxicity study (LC-50) in the carp: determination of the stability of ORASOL ORANGE G under test conditions and actual test concentrations using a High Performance Liquid Chromatographic method.

Determination of stability in a pretest revealed that at a nominal concentration of 10 mg/l, ORASOL ORANGE G was stable under test conditions for at least 96 hours.

Analysis of the samples taken during the main study revealed that no accurate results could be obtained due to the observed variability in the analytical method. During the exposure period the actual concentration analyzed decreased significantly.

PREFACE

GENERAL

Title	96-hour acute toxicity study (LC-50) in the carp. Determination of test concentrations.
Sponsor	CIBA-GEIGY AG Ch-4002 Basel Switzerland
Monitoring Scientist	Dr. A. von Schulthess
Testing Facility	RCC NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch, The Netherlands
RCC NOTOX Project Number	007919
Test substance	ORASOL ORANGE G

PROJECT STAFF

Study Director	Drs. M. Bogers (RCC NOTOX B.V.)
Principal Scientist	Ir. J.M. Cardinaals (RCC NOTOX B.V.)

SCHEDULE

Start of the study	September 05, 1989
Completion of the study	October 20, 1989

ARCHIVING

RCC NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.

PURPOSE

The purpose of the study was to determine the stability and the test concentrations of ORASOL ORANGE G in test medium.

MATERIALS AND METHODS

TEST SYSTEM

Test medium	Tap water
Nominal concentrations	Range finding: 0 mg/l, 0.1 mg/l, 1.0 mg/l and 10 mg/l Main study: 0 mg/l, 0.01 mg/l, 0.056 mg/l and 0.32 mg/l

TEST SUBSTANCE

Identification	ORASOL ORANGE G
Description	Orange solid
Batch Number	216096.89
Purity	45-55% Orasol Orange G new type Comp. I 55-45% Orasol Orange G new type Comp. II
Storage conditions	At 20°C in the dark
Stability of test substance	Stable, > 5 years at storage conditions Expiry date: 31-12-1993
Safety precautions	Gloves and goggles were used to assure personnel health and safety. All practical handlings were performed in a fume cupboard.

SAMPLE HANDLING

Storage	All samples to be analyzed for project 007919 were analyzed immediately after sampling.
Sampling date	Range finding study: Duplicate samples were taken on 02-10-89 (t=0h), 03-10-89 (t=24h) and on 06-10-89 (t=96h). Analyses were performed on 03-10-89 (t=0h and t=24h samples) and on 04-10-89 (t=96h samples). For practical reasons, the t=0h samples were stored overnight at room temperature in the dark, prior to analysis. Main study: Duplicate samples were taken and analyzed on 16-10-89 (t=0h and t=2h), 17-10-89 (t=24h), 18-10-89 (t=48h) and on 20-10-89 (t=96h).
Pretreatment	The 10 mg/l samples were diluted 10 times using tap-water. All remaining samples were analyzed without further dilution.

QUANTITATIVE ANALYSIS

Calibration solutions	Two independently prepared calibration solutions of ORASOL ORANGE G in ethanol (pro-analysis, Merck) were used each day of analysis to calibrate the analytical method. These solutions were diluted with tap-water prior to analysis, except for the analysis of the t=0h samples of the range finding study. Suitable calibration solutions for these samples were prepared and diluted on 02-10-89, and stored overnight under identical conditions as the samples.
Method of chemical analysis	High Performance Liquid Chromatographic method (HPLC).
Detection limit	By further dilution of calibration solutions. the detection limit was determined.

HPLC CONDITIONS

Column	length = 250 mm, inner diameter = 4 mm
Stationary phase	LiChrosorb RP-18 (Merck, FRG).
Mobile phase	40% Milli-Q water (Millipore Corp., Bedford, Mass., U.S.A.). 60% methanol (LiChrosolv, Merck, FRG) 0.2% ethylamine (70% EGA chemie, Steinheim/Albuch, FRG)
Flow	1 ml/min
Detection	UV, at 257 nm
Retention time	approximately 5.7 minutes
Injection volume	50 µl
Instrumentation:	
pump:	LDC Milton Roy constaMetric 3000 or Waters 510 HPLC pump
detector:	LDC Milton Roy spectroMonitor 3100
sampling system:	Programmable Multifunctional Injection System (PROMIS, SPARK, Holland) or Waters 712 WISP autosampler
integrator	Spectra Physiscs SP 4290 or SP 4400

Typical HPLC chromatograms are shown in Figure 1 (calibration solution), 2 (sample) and 3 (blank sample).

RESULTS

The results obtained for the concentrations of ORASOL ORANGE G in the test medium (range finding study) are shown in Table 1.

The detection limit was determined to be 0.005 mg/l.

Table 1 Results of the determination of the concentration of ORASOL ORANGE G in test medium (range finding study).

Date of preparation	Date of analysis	Concentration [mg/l][1] prepared	analyzed[2]
02-10-89	03-10-89 (t=0h)	10	9.8 /9.5 (98%/97%)
		1	0.9 /0.7 (90%/70%)
		0.1	0.03/0.03 (30%/30%)
02-10-89	03-10-89 (t=24h)	10	9.1 /9.5 (91%/95%)
		1	0.5 /0.6 (50%/60%)
		0.1	0.03/0.02 (30%/20%)
02-10-89	06-10-89 (t=96h)	10	9.5 /9.5 (95%/95%)
		1	0.6 /0.6 (60%/60%)
		0.1	0.02/0.02 (20%/20%)

1 Values between brackets represent concentration analyzed relative to concentration prepared.
2 Results of duplicate samples.

In all blank samples, no test substance was observed (< 0.005 mg/l).

During both days of analysis, a relatively large inter and intra variability (up to approximately 10%) in both the calibration solutions and the samples was observed, specially at the lower concentrations. Thus, the conclusions derived from these analyses must be handled with care. From Table 1, it can be concluded that at a concentration of 10 mg/l, ORASOL ORANGE G is stable in test medium for at least 96 hours and at this level the concentration analyzed was in agreement with the concentration prepared. Both the 1 mg/l and 0.1 mg/l concentrations show a tendency to decline in time, and furthermore differ significantly from the concentrations prepared.

The results of the analyses performed during the main study are shown in Table 2.

Table 2 Results of the determination of the concentration of ORASOL ORANGE G in test medium (main study).

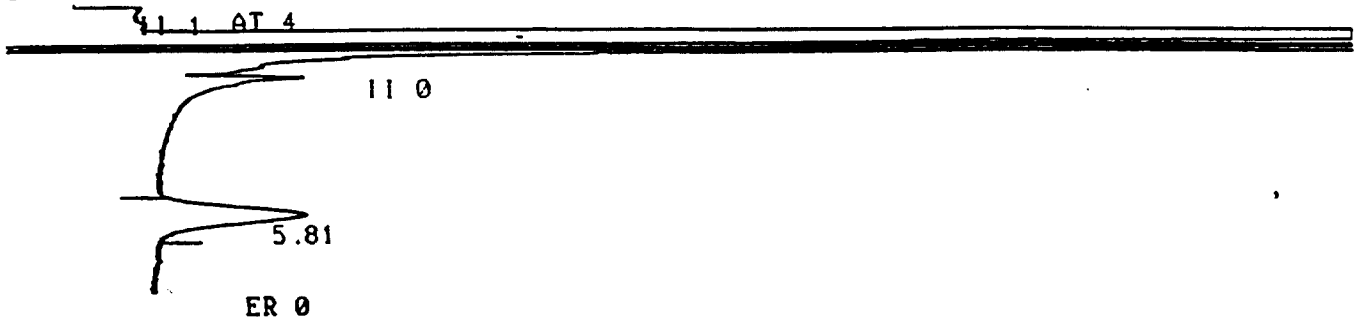
Date of sampling	Date of analysis	Concentration [mg/l][1] prepared	analyzed[2]	
16-10-89 (t=0h)	16-10-89	0.32	0.1 -0.3	(30-100%)
		0.056	0.02-0.04	(35-70%)
		0.010	<0.005	(<50%)
16-10-89 (t=2h)	16-10-89	0.32	0.2 -0.4	(60%-125%)
		0.056	0.03-0.06	(50%-110%)
		0.010	<0.005	(<50%)
17-10-89 (t=24h)	17-10-89	0.32	0.1 -0.2	(30-60%)
		0.056	0.02-0.03	(35-55%)
		0.010	<0.005	(<50%)
18-10-89 (t=48h)	18-10-89	0.32	0.1 -0.2	(30%-60%)
		0.056	0.006	(10%)
		0.010	<0.005	(<50%)
20-10-89 (t=96h)	20-10-89	0.32	0.1 -0.2	(30%-60%)
		0.056	<0.005	(<10%)
		0.010	<0.005	(<50%)

[1] Values between brackets represent concentration analyzed as percentage of nominal concentration.
[2] Results of the duplicate samples are given as a range, due to the relatively large inter and intra variability in the method of analysis.

In Table 2 the results of the analyses are presented as ranges. This to indicate that no accurate results could be obtained due to the observed variability in the analytical method. Thus, conclusions drawn from Table 2 must be handled with care.
The concentration of ORASOL ORANGE G detected in most samples was significantly lower than the concentration prepared. Furthermore, the actual concentrations analyzed at nominally 0.32 mg/l and 0.056 mg/l decreased with time.

Figure 1 Typical HPLC chromatogram of a calibration solution (0.1145 mg/l). Chromatographic conditions used are outlined in this report.

CHANNEL A INJECT 20-10-89 12:26:31 STORED TO BIN # 46



ER 0
DATA SAVED TO BIN # 46

INPUT OVERRANGE AT RT= 1.27

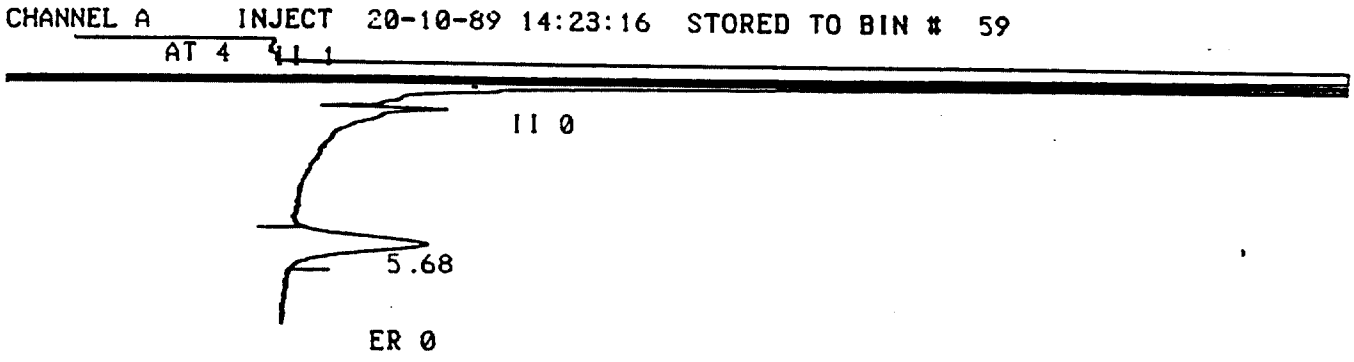
2195 007919 20-10-89 12:26:31 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 350 INDEX 195 BIN 46

ANALYST: AVK

PEAK#	AREA%	RT	AREA BC
1	100.	5.81	26104 01
TOTAL	100.		26104

Figure 2 Typical HPLC chromatogram of a sample (sampling date: October 20, 1989; nominal concentration: 0.32 mg/l). Chromatographic conditions used are outlined in this report.



DATA SAVED TO BIN # 59

INPUT OVERRANGE AT RT= 1.27

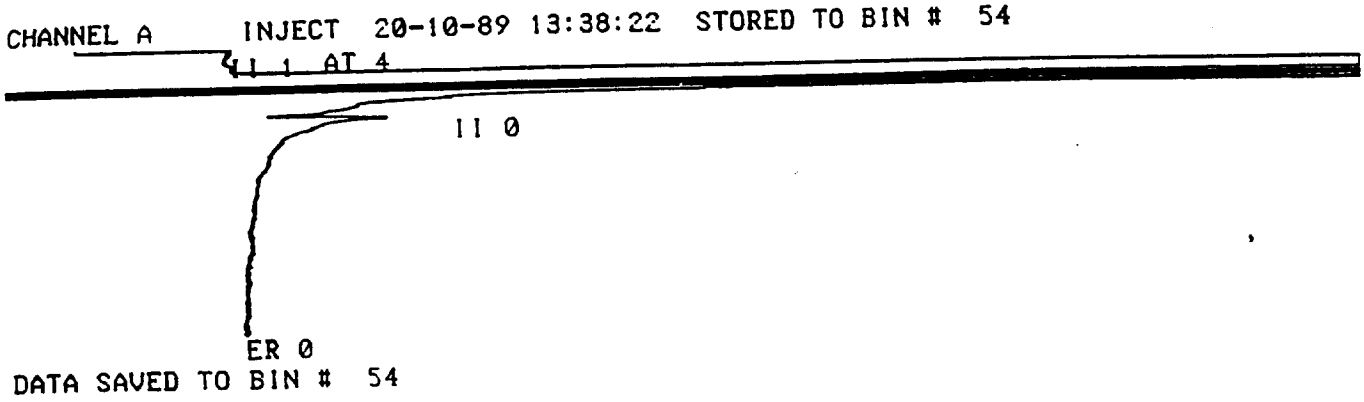
2195 007919 20-10-89 14:23:16 CH= "A" PS= 1

FILE 1. METHOD 0. RUN 363 INDEX 208 BIN 59

ANALYST: AVK

PEAK#	AREA%	RT	AREA BC
1	100.	5.68	22774 01
TOTAL	100.		22774

Figure 3 Typical HPLC chromatogram of a blank sample. Chromatographic conditions used are outlined in this report.



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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

APR 2 4 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

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EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure 13348A

Triage of 8(e) Submissions

Date sent to triage: 12/14/95

(NON-CAP) CAP

Submission number: 13348A

TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO (AQUATO)

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

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Notes: 2 sided

Contractor reviewer : LPS **Date:** 4/14/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0395 - 13348 SEQ. A

TYPE: INT. SUPP. FLWP

SUBMITTER NAME: Ciba - Geigy Corporation

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 03/02/95 OTS DATE: 03/03/95 CSRAD DATE: 03/15/95

CHEMICAL NAME:	CAS#
Orasol Orange G	none
	71839-88-8
	85203-44-7

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES	YES (DROP/REFER)	Carp	LOW	imported pigment sold Commercial	
CAS SR	NO	NO (CONTINUE)		MED	organic solvent based ink	in U.S.
	UNDETERMINED	REFER		HIGH		

COMMENTS: Non-Cap